PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ES/13310.4	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/CA 03/01429	International filing date *(day/month/year)* 19.09.2003	Priority date *(day/month/year)* 20.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/00		
Applicant MEDINNOV, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.04.2004	11.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Jenkins, G Telephone No. +31 70 340-2608

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA 03/01429

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-29 as originally filed

Claims, Numbers

1-45 received on 13.09.2004 with letter of 07.09.2004

Drawings, Sheets

1/21-21/21 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-8,11-26,30-34,37-45
	No:	Claims	1-3,9,10,27-29,35,36
Inventive step (IS)	Yes:	Claims	23-26
	No:	Claims	1-22,27-45
Industrial applicability (IA)	Yes:	Claims	1-45
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The following documents (D1-D2) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: PLEGGE ET AL: 'Analysis of ternary mixtures with a single dynamic microbial sensor and chemometrics using a nonlinear multivariate calibration', ANAL CHEM, 01. June 2000, vol. 72, no. 13, pages 2937 to 2942,

D2: US 5312590 A 1994.05.17 998, vol. 17, no. 6-7, pages 1111 to 1128.

2 NOVELTY

2.1 The present application does not meet the requirements of Article 33(1) PCT, because the subject-matter of claim 1-3,9,10,27-29,35,36 is not new in the sense of Article 33(2) PCT.

2.1.1 D2 discloses (the references in parentheses applying to this document): a device suitable for measuring the concentration of two related analytes that are substrates for a common enzyme comprising a support base (figure 3); a mixed electrode system comprising a platinum working electrode, a platinum auxiliary electrode, and a silver reference electrode (column 4, lines 22-25,64-69; claim 11); an enzymatic reaction means (glucose oxidase: column 7, lines 9-24); a detector (figure 9); a data processor capable of converting amplified signals into numerical data representing the concentration of two analytes; a layer of a TTF/Nafion on which glucose oxidase is bound (column 7, lines 9-24); a protective polycarbonate membranae (column 7, lines 9-24), and a reagent well (figure 3). Ferrocene mediators are also disclosed (column 2, lines 25-27). In accordance with the PCT Guidelines Section IV 5.23, 12.05, the intended use of the apparatus cannot be used here to establish novelty (i.e. placing the enzyme in contact with a liquid sample containing two related analytes), since the apparatus of D2 could also be contacted with two related analytes (e.g. glucose

and mannose) and its processor used to convert the electronic signals into the concentration of each anaiyte. The subject-matter of claims 1-3,9,10,27-29,35,36 is therefore not new (Article 33(2) PCT).

2.1.2 The subject-matter of method claims 4-8,11-26,30-34,37-45 is considered novel (Article 33(2) PCT).

3 INVENTIVE STEP

3.1 The present application does not meet the requirements of Article 33(1) PCT, because the subject-matter of claims 4-8,11-22,30-34,37-45 does not involve an inventive step in the sense of Article 33(3) PCT.

3.1.1 The subject-matter of claims 4-8,11-22 merely adds routine modification options to the subject-matter of claim 1 and is therefore obvious to a person skilled in the art. For this reason the subject-matter of claims 4-8,11-22 does not involve an inventive step in the sense of Article 33(3) PCT.

3.1.2 The subject-matter of claims 30-34,37-45 merely adds routine modification options to the subject-matter of claim 27 and is therefore obvious to a person skilled in the art. For this reason the subject-matter of claims 30-34,37-45, does not involve an inventive step in the sense of Article 33(3) PCT.

3.2 The subject-matter of claims 23-26 is considered inventive under Article 33(3) PCT.

3.2.1 The subject-matter of claim 23 is considered inventive under Article 33(3) PCT. Here, D1 is considered the closest prior art. This document discloses (the references in parentheses applying to this document): a method for simultaneously measuring the concentration of acetate, L-lactate and succinate involving a) reacting a plurality of reference samples having known ~~concentrations and proportions of said related analytes with microorganisms (p.~~ 2939, column 1, paragraph 2), b) establishing a kinetic profile having at least two points for each of said plurality of reference samples (p. 2940), c) reacting a

test sample with microorganisms and determining concentrations of related components (table 2).

3.2.1.1 The additional technical feature of claim 23 over D1 is that the simultaneous multi-species analyte determination is achieved using a single enzyme, rather than a mixture of enzymes (e.g. microorganism cells).

3.2.1.2 The technical effect associated with this modification is a simpler assay.

3.2.1.3 The problem to be solved by the present invention may therefore be regarded as the provision of a simpler assay for simultaneously measuring the concentration of two related analytes.

3.2.1.4 The solution to this problem is to use a single enzyme, rather than a mixture of enzymes.

3.2.1.5 The solution to the problem is not suggested or derivable in an obvious way from the prior art. Therefore, the subject-matter of claim 23 is inventive in the sense of Article 33(3) PCT. Claims 24-26 are dependent on claim 23, and their subject-matter is therefore also inventive (Article 33(3) PCT),

4 INDUSTRIAL APPLICABILITY

4.1 The subject-matter of claims 1-45 is industrially applicable in the field enzyme electrodes (Article 33(4) PCT).

CLAIMS:

1. An analyzer for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

(a) an enzymatic reaction monitoring component including a support base, a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base, and an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample;

(b) a detector including a sensor, said detector being connected to said enzymatic reaction monitoring component and capable of continuously detecting and amplifying said electronic signal to produce amplified signals; and

(c) a data processor capable of converting the amplified signals into numerical data representative of the concentration of each of said two related analytes.

2. An analyzer as defined in claim 1, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

3. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound a layer including said enzyme, said layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

4. An analyzer as defined in claim 3, wherein said permeable polymer is selected from the group consisting of polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

5. An analyzer as defined in claim 4, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

6. An analyzer as defined in claim 5, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

7. An analyzer as defined in claim 6, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

8. An analyser as defined in claim 7, wherein said enzymatic reaction monitoring component is a disposable electrode.

9. An analyzer as defined in claim 2, wherein said enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

10. An analyzer as defined in claim 9, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

11. An analyzer as defined in claim 10, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

12. An analyzer as defined in claim 11, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

13. An analyser as defined in claim 12, wherein said enzymatic reaction monitoring component is a permanent electrode.

14. An analyzer as defined in claims 8 and 13, wherein said enzyme is an oxidase.

15. An analyser as defined in claim 14, wherein said oxidase is alcohol oxidase.

16. An analyzer as defined in claim 15, wherein said related analytes are methanol and ethanol.

17. An analyzer as defined in claim 16, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

18. An analyzer as defined in claim 17, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

19. An analyzer as defined in claim 18, wherein said support base is composed of plastic.

20. An analyzer as defined in claim 8, wherein said analyzer is a portable analyzer.

21. An analyzer as defined in claim 13, wherein said analyzer is a non-portable analyzer.

22. An analyzer as defined in claims 20 and 21, for use in point-of-care units, in laboratories, in police services, in forensic applications and in industrial applications.

23. A method for simultaneously detecting and measuring the concentration of ~~at least~~ two related analytes in a sample, said related analytes being substrates for a common enzyme, wherein said enzyme reacts with said related analytes following specific different reaction kinetics, and wherein said method comprises:

(a) reacting a plurality of reference samples having known concentrations and proportions of said related analytes, said proportions ranging from 0 to 100% of a first analyte to 100% to 0% of another related analyte, with said enzyme;

(b) establishing a kinetic profile having at least two points for each of said plurality of reference samples; and

(c) reacting a test sample comprising an unknown concentration and proportion of said related analytes with said enzyme and determining the concentration of said related compounds in said test sample using said established kinetic profiles.

24. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using multiple regression analysis of said kinetic profile.

25. A method as defined in claim 23, wherein said unknown concentration of said related analytes is established using reaction kinetics equations.

26. A method as defined in claim 24 and 25, wherein said related analytes are methanol and ethanol.

27. An enzymatic reaction monitoring component for simultaneously detecting and measuring the concentration of two related analytes, said analytes being substrates for a common enzyme, comprising:

(a) a support base;

(b) a mixed electrode system consisting of a working electrode, an auxiliary electrode and a reference electrode, said mixed electrode system being supported by said support base; and

(c) an enzymatic reaction means incorporating said enzyme, said enzymatic reaction means being disposed on said mixed electrode system; whereby, when said enzymatic reaction means is placed in contact with a liquid sample containing said two related analytes, said two related analytes chemically react with said enzyme to produce an electronic signal directly related to the concentration of each of said two related analytes in said liquid sample.

28. An enzymatic reaction monitoring component as defined in claim 27, wherein said working electrode and said auxiliary electrode are composed of platinum, and wherein said reference electrode is composed of silver.

29. An enzymatic reaction monitoring component as defined in claim 28, wherein said enzymatic reaction means comprises a layer of a permeable polymer on which is bound an enzyme layer, said enzyme layer being deposited on said mixed electrode system, and a protective membrane impregnable with a buffer solution and reagents capable of promoting said enzymatic reaction, said protective membrane being disposed over said layer of a permeable polymer.

30. An enzymatic reaction monitoring component as defined in claim 29, wherein said permeable polymer is selected from the group consisting of

polylysine, poly(4-styrene sulfonate), polyethylene glycol, perfluorosulfonic acid polymers and agarose.

31. An enzymatic reaction monitoring component as defined in claim 30, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

32. An enzymatic reaction monitoring component as defined in claim 31, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

33. An enzymatic reaction monitoring component as defined in claim 32, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

34. An enzymatic reaction monitoring component as defined in claim 33, wherein said enzymatic reaction monitoring component is a disposable electrode.

35. An enzymatic reaction monitoring component as defined in claim 28, wherein enzymatic reaction means comprises a reagent well capable of receiving a buffer solution including said enzyme, said liquid sample, and optionally reagents capable of promoting said enzymatic reaction.

36. An enzymatic reaction monitoring component as defined in claim 35, wherein said reagents include electron transfer reagents selected from the group consisting of p-phenylenediamine, peroxidase and ferrocene derivatives.

37. An enzymatic reaction monitoring component as defined in claim 36, wherein said ferrocene derivatives include ferrocene dicarboxylic acid, and ferrocene monocarboxylic acid.

38. An enzymatic reaction monitoring component as defined in claim 37, wherein said buffer solution is selected from the group consisting of phosphates, saline phosphate buffers (phosphates + NaCl), TRIS-HCl, Hepes, with or without EDTA, and a wetting agent such as SDS, Triton X-100 and Tween 20.

39. An enzymatic reaction monitoring component as defined in claim 38, wherein said enzymatic reaction monitoring component is a permanent electrode.

40. An enzymatic reaction monitoring component as defined in claims 34 and 39, wherein said enzyme is an oxidase.

41. An enzymatic reaction monitoring component as defined in claim 40, wherein said oxidase is alcohol oxidase.

42. An enzymatic reaction monitoring component as defined in claim 41, wherein said related analytes are methanol and ethanol.

43. An enzymatic reaction monitoring component as defined in claim 42, wherein said liquid sample is a biological specimen selected from the group consisting of saliva, blood or serum.

44. An enzymatic reaction monitoring component as defined in claim 43, wherein said support base is composed of any suitable material capable of supporting said mixed electrode system.

45. An enzymatic reaction monitoring component as defined in claim 44, wherein said support base is composed of plastic.